
# Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection



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# Antiretroviral Drug-Resistance Testing (Updated August 11, 2011)

| Panel's Recommendations |
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| • Antiretroviral (ARV) drug-resistance testing is recommended before initiation of therapy in all treatment-naive children **(AII)**. Genotypic resistance testing is preferred for this purpose **(AIII)**. |
| • ARV drug-resistance testing is recommended before changing therapy for treatment failure **(AI*)**. |
| • Resistance testing in the setting of virological failure should be obtained while the patient is still on the failing regimen or within 4 weeks of discontinuing the regimen **(AII*)**. |
| • Phenotypic resistance testing should be used (usually in addition to genotypic resistance testing) for patients with known or suspected complex drug resistance mutation patterns, which generally arise after virologic failure of successive ARV therapy regimens **(BIII)**. |
| • The absence of detectable resistance to a drug does not ensure that use of the drug will be successful, especially if the ARV agent shares cross resistance with drugs previously used. In addition, current resistance assays are not sensitive enough to fully exclude the presence of resistant virus. Thus, previously used ARV agents and previous resistance test results should be reviewed when making decisions regarding the choice of new agents for patients with virologic failure **(AII)**. |
| • Viral coreceptor (tropism) assays should be used whenever the use of a CCR5 antagonist is being considered **(AI*)**. Tropism assays should also be considered for patients who demonstrate virologic failure while receiving therapy that contains a CCR5 antagonist **(AI*)**. |
| • Consultation with a specialist in pediatric HIV infection is recommended for interpretation of resistance assays when considering starting or changing an ARV regimen in a pediatric patient **(AI*)**. |

## HIV Drug-Resistance and Resistance Assays

HIV replication is a continuous process in most untreated patients, leading to the daily production of billions of viral particles. The goal of antiretroviral therapy (ART) is to suppress HIV replication as rapidly and fully as possible, indicated by a reduction in plasma HIV RNA to below the limit of detection of the most sensitive assays available (HIV RNA <40–80 copies/mL). Unfortunately, mutations in HIV RNA readily arise during viral replication because HIV reverse transcriptase (RT) is a highly error-prone enzyme. Consequently, ongoing replication in the presence of ARV drugs readily and progressively selects for strains of HIV with mutations that confer drug resistance.

Drug-resistance detection methods vary depending on the class of ARV agents. Viral coreceptor (tropism) assays have been successfully employed to detect virus with tropism that will (CCRR5 tropism) or will not (CXCR4 or mixed tropism) be blocked by CCR5 antagonists. Both genotypic assays and phenotypic assays are used to detect the presence of virus that is resistant to inhibitors of the HIV RT, integrase, or protease (PR). Clinical experience with testing for viral resistance to other agents is more limited, but genetic mutations associated with resistance to integrase strand transfer inhibitors (INSTIs) have been identified, and a commercial phenotypic assay is available for evaluation of resistance to the fusion inhibitor enfuvirtide. Experience with the use of commercially available genotypic and phenotypic assays in the evaluation of drug resistance in patients infected with non-B subtypes of HIV[1] is also limited.

### *Genotypic Assays*

Genotypic assays for resistance to RT, PR, and INSTIs are based on polymerase chain reaction (PCR) amplification and analysis of the RT, PR, and integrase coding sequences present in HIV RNA extracted from plasma. Genotypic assays can detect resistance-associated mutations in plasma samples containing approximately 1,000 copies/mL or more of HIV RNA and results are generally available within 1–2 weeks of sample collection[2]. Interpretation of test results requires knowledge of the mutations selected by different ARV drugs and of the potential for cross resistance to other drugs conferred by certain mutations. For some drugs, the genetic barrier to the development of resistance is low, and a single nucleotide mutation is enough to confer high-level resistance sufficient to remove any clinical utility of the drug. This is exemplified by resistance to nevirapine resulting from mutations in the HIV RT. Other mutations lead to drug resistance but simultaneously impair HIV replication. Clinically useful activity of the ARV agent may therefore remain, as demonstrated by evidence of continued clinical benefit from lamivudine in individuals with evidence of the high-level resistance engendered by the M184V RT mutation[3]. Other mutations have little direct effect on resistance but arise during HIV evolution to high-level resistance or improve the replication of virus-bearing mutations that confer high-level resistance to an ARV agent.

The International Antiviral Society-USA (IAS-USA), the Los Alamos HIV Drug Resistance Database, and the Stanford University HIV Drug Resistance Database maintain lists of significant resistance-associated mutations relevant to currently available ARV drugs (see http://www.iasusa.org/resistance_mutations, http://hiv-web.lanl.gov, or http://hivdb.stanford.edu). A variety of online tools that take into account the ability of some mutations selected by one drug to cause partial or full cross resistance with other drugs are now available to assist the provider in interpreting genotypic test results. Although the response to ART in children and adolescents is not always predicted by the results of genotypic resistance assays, clinical trials in adults have demonstrated the benefit of resistance testing combined with consultation with specialists in HIV drug resistance in improving virologic outcomes[2,4-10]. Given the potential complexity of interpretation of genotypic resistance, it is recommended that clinicians consult with a specialist in pediatric HIV infection for assistance in the interpretation of genotypic results and design of an optimal new regimen.

### *Phenotypic Assays*

Phenotypic resistance assays provide a more direct assessment of the impact on viral replication of mutations that are present among an individual's HIV variants. As they are most often performed, phenotypic assays involve PCR amplification of the RT, integrase, PT, or other HIV gene sequences from patient plasma and insertion of those amplified patient sequences into the backbone of a laboratory strain of HIV. Replication of this recombinant virus at different drug concentrations is monitored by expression of a reporter gene and is compared with replication of a reference HIV strain. The drug concentration that inhibits viral replication by 50% (i.e., the median inhibitory concentration, or IC50) is calculated, and the ratio of the IC50 of test and reference viruses is reported as the fold increase in IC50 (i.e., fold resistance change). Automated, recombinant phenotypic assays that can produce results in 2–3 weeks are commercially available; however, they are more costly than genotypic assays.

Analytic techniques have also been developed to use the genotype to predict the likelihood of a drug-resistant phenotype. This bioinformatic approach, currently applicable for RT and PI resistance only, matches the pattern of mutations obtained from the patient sample with a large database of samples for which both genotype and phenotype are known. Thus, the sample is assigned a predicted phenotype susceptibility (or "virtual phenotype") based on the data from specimens matching the patient's genotype. The primary limitations of this approach are that its predictive power depends upon the sensitivity of the

genotypic methods used and the number of matched phenotypic and genotypic assays available for data analysis, which may be limited for newer drugs.

### *Tropism (Viral Coreceptor Usage) Assays*

HIV enters cells by a complex multistep process that involves sequential interactions between the HIV envelope protein molecules and the CD4 receptor, then with either the CCR5 or CXCR4 coreceptor molecules, culminating in the fusion of the viral and cellular membranes. Viruses in the majority of untreated individuals, including infants and children infected by mother-to-child transmission (MTCT) of HIV, are initially CCR5 tropic. However, a shift in coreceptor tropism often occurs over time, from CCR5 usage to either CXCR4 or both CCR5 and CXCR4 tropism (dual- or mixed-tropic; D/M-tropic). ARV-treated patients with extensive drug resistance are more likely to harbor detectable CXCR4- or D/M-tropic virus than untreated patients with comparable CD4 T-cell counts[11].

Resistance to CCR5 antagonists is currently detected using the specialized phenotypic assay methods Phenoscript (VIRalliance) and Trofile (Monogram Biosciences, Inc). These assays involve the generation of recombinant viruses bearing patient-derived envelope proteins (gp120 and gp41). The relative capacity of these pseudoviruses to infect cells bearing the cell surface proteins CCR5 or CXCR4 is quantified based on the expression of a reporter gene. The Trofile assay takes about 2 weeks to perform and requires a plasma viral load ≥1,000 copies/mL. The initial version of the Trofile assay used during the clinical trials that led to the licensure of maraviroc was able to detect CXCR4-tropic virus with 100% sensitivity when present at a frequency of 10% of the plasma virus population but only 83% sensitivity when the variant was present at a frequency of 5%. In initial clinical trials of CCR5 antagonist drugs, this sensitivity threshold was not always sufficient to exclude the presence of clinically meaningful levels of CXCR4- or D/M-tropic virus in patients initiating a CCR5 inhibitor-based regimen. A newer version of the Trofile assay with improved sensitivity able to detect CXCR4- or D/M-tropic virus representing as little as 0.3% of the plasma virus is now available[12-13]. A genotypic assay to detect mutations associated with CXCR4- or D/M-tropic virus (Trofile-DNA) is also available. Although experience with these genotypic assays is somewhat limited, evidence that they may be useful substitutes for phenotypic tropism assays does exist[14]. Any indication of CXCR4 tropism is a contraindication to the use of the CCR5 antagonists as part of a therapeutic regimen. Coreceptor use assays should be performed before the use of a CCR5 inhibitor and may be considered in patients exhibiting virologic failure on a CCR5 inhibitor such as maraviroc. Because genotypic tropism assays can be performed on peripheral blood DNA, they may be useful when a change to a regimen containing a CCR5 antagonist is being considered for an individual with an undetectable plasma viral load.

### *Limitations of Current Resistance and Tropism Assays*

Limitations of the genotypic, phenotypic, and phenotype-prediction assay approaches include lack of uniform quality assurance testing and high cost. In addition, drug-resistant variants are likely to exist at low levels in every HIV-infected patient. Drug-resistant viruses that constitute <10%–20% of the circulating virus population may not be detected by any of the currently available commercial assays[15]. Consequently, a review of the past use of ARV agents is important in making decisions regarding the choice of new agents for patients with virologic failure.

Although drug resistance may be detected in infants, children, and adults who are not receiving therapy at the time of the assay, loss of detectable resistance and reversion to predominantly wild-type virus often occur in the first 4–6 weeks after ARV drugs are stopped[16-18]. As a result, resistance testing is of greatest value when performed before or within 4 weeks after drugs are discontinued. The absence of de-

tectable resistance to a drug at the time of testing does not ensure that future use of the drug will be successful[19], especially if the agent shares cross resistance with drugs previously used. It may be prudent to repeat resistance testing if an incomplete virological response to a new treatment regimen is observed in an individual with prior treatment failure(s) (see Antiretroviral Treatment Failure in Infants, Children, and Adolescents).

### *Use of Resistance Assays in Determining Initial Treatment*

MTCT and behavioral transmission of drug-resistant HIV strains have been well documented and are associated with suboptimal virologic response to initial ART[20-24]. Drug-resistant variants of HIV may persist in infected infants[25] for months after birth and impair the response to ART[26]. Consequently, ARV drug-resistance testing is recommended prior to initiation of therapy in all treatment-naive children. Genotypic testing is preferred in this setting because it may reveal the presence of both resistance mutations and polymorphisms that facilitate the replication of drug-resistant virus.

### *Use of Resistance Assays in the Event of Virologic Failure*

Several studies in adults[2,4-10] have indicated that early virologic responses to salvage regimens were improved when results of resistance testing were available to guide changes in therapy, compared with responses observed when changes in therapy were guided only by clinical judgment. Although not yet confirmed in children[27], resistance testing appears to be a useful tool in selecting active drugs when changing ARV regimens in cases of virologic failure. Resistance testing also can help guide treatment decisions for patients with suboptimal viral load reduction because virologic failure in the setting of combination antiretroviral therapy (cART) may be associated with resistance to only one component of the regimen[1]. Poor adherence should be suspected when no evidence of resistance to a failing regimen is identified (see Antiretroviral Treatment Failure in Infants, Children, and Adolescents).

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